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Morphologic Changes in Collagen Fibers After 830 nm Diode Laser Welding

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Background and Objective: **The mechanism of laser tissue welding is elusive, but collagen transitions are somehow involved. Collagen fiber modifications observed after 830 nm diode laser welding are presented in this study.**
Study Design/Materials and Methods: **A 830 nm diode laser assisted longitudinal aortorrhaphy was performed on 37 Wistar rats, with shots of 0.5 W in power, 8 sec in duration and 250 W/cm^2 in irradiance. Energy utilized ranged from 400–550 J/mm^2 for 1 cm-length of anastomosis. After laser welding, histological modifications in collagen fibers were observed through optic, scanning electron, and electron microscopic examination.**
Results: **After laser welding, collagen fibers lost a proportion of birefringence. Under electron microscope, the different changes in collagen fibers were visualized being either fused, "roped," swollen, or dissolved, surrounded by normal ones situated in the same zone.**
Conclusion: **These data suggest that diode laser heating denatured part of the collagenic fibers, and that these morphologic changes play an important role in laser welding. Lasers Surg. Med. 21:438–443, 1997.** **© 1997 Wiley-Liss, Inc.**

Key words: diode laser; laser welding; collagen fiber modification

INTRODUCTION

At the present time, mechanisms of laser vessel welding remain unknown. Several studies have attempted to define the responsibility of collagen alterations in the mechanism of vascular sealing, and demonstrated the molecular changes observed after laser heating [1–5].

The aim of this study was to present the optical, electron, and scanning electron microscopic findings of 830 nm diode laser-assisted aortorrhaphy in the Wistar rat, and to observe the morphologic changes of collagen fibers.

MATERIALS AND METHODS

Longitudinal aortorrhaphy (aortotomy) was performed on 37 Wistar rats (weight range 300–540 g; mean weight 382 g). The animals were anesthetized with a solution of intramuscular Vetranquil® (0.4 mg/kg body weight) and Ketamine (80 mg/kg body weight). All operative procedures were carried out using standard microsurgical technique under an operating microscope (Zeiss OPMI 1, Berkochen, Germany). The aorta was dissected and isolated with section of the electrocoagulated iliolumbar and testicular arteries, and then mobilized on a distance of 10–15 mm in the region between the renal vessels and the aortic bifurcation. The vessel size ranged from 1.5–2.0 mm in diameter, and 100–150 μm in thickness. After the blood flow was occluded with

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two microclamps, a 5–6 mm longitudinal incision was made in the anterior wall of the vessel. The aortic lumen was irrigated with 0.9% saline solution in order to remove intravascular blood. Excess saline solution was then wiped off with a compress, and the vessel was dry before the laser irradiation. Interrupted 10-0 Ethilon® BV 70-3 (Ethnor Society, Neuilly, France) sutures were placed at 2–3 mm intervals to provide adequate vessel edge approximation. Three or four sutures were needed, and they were left in place after the operation. No anticoagulation was used.

Laser Application

Laser-assisted anastomosis was performed with a 830 nm continued wavelength diode laser device (prototype, Swiss Federal Institute of Technology, Lausanne, Switzerland). Laser energy was delivered through an 100 μm diameter optic fiber and transmitted to the vessel surface through a micromanipulator (prototype: our laboratory) coupled to the operating microscope, giving a spot size of 0.5 mm and guided by a diode red light (670 nm). The laser energy leaving the micromanipulator was measured by a powermeter (Gentec, TPM-330, Quebec, Canada). Laser treatment was achieved using juxtaposed shots. Each shot was 0.5 W in power, 8 sec in duration, and 250 W/cm^2 in irradiance. The lasing parameters and suture spacing were chosen from our previous experiments of diode laser vascular welding [6,7] and preliminary experiments. Ten to 15 shots were needed for each anastomosis. On completion of the laser fusion, areas that did not seal were closed by addition laser irradiation (one to two shots per an anastomosis). Energy utilized ranged between 400–550 J/mm^2 per 1 cm-length of anastomosis. The accuracy of the weld and the luminal patency of each anastomosis was controlled by intraoperative examination and by Doppler (Prototype 1190 DPP01, Laboratory of Medical Biophysics, Tours, France) spectral analysis.

The laser anastomosed vessels were harvested immediately, day 3 and day 10 (Table 1). Among the group harvested immediately after laser welding, six aortas were fixed with 2.5% glutaraldehyde just before stopping laser irradiation. The samples were harvested for scanning electron microscopic and electron microscopic examinations (n = 3, for each) in order to observe the morphology of collagen's fibers in the heated condition.

TABLE 1. The Harvested Dates and Numbers of Samples for Light Microscopy (LM), Scanning Electron Microscopy (SEM), and Electron Microscopy (EM)

Date	LM	SEM	EM	Total
Immediately	9	5	7	22
Day 3	3	3	4	8
Day 10	5	2	2	7
Total	22	10[a]	13[a]	37

[a]Some specimens were divided into two parts for SEM and EM simultaneously.

Histological Technique

Light microscopy. Samples for light microscopy (LM), were fixed in 5% formalin, dehydrated in methanol, cleared in methyl benzoate and benzene, and impregnated with paraffin wax. Transversal sections (6 μm thick) were performed and lames stained with four methods: 1) van Gieson and Heidenhain's azan stains for common study of collagen tissue; 2) picrosirius red F3BA stain for detecting the natural birefringence of collagen under polarized light. The birefringence disappeared when collagenous fibers were denatured; 3) Verhoeff-van Gieson stain for simultaneous characterization of elastic fibers and collagenous fibers; and 4) haematoxylin-eosin stain.

Scanning electron microscopy. Samples for scanning electron microscopy (SEM), were perfused with 0.9% saline solution, fixed in 2.5% glutaraldehyde, and washed in cacodylate buffer (pH 7.4). After dehydration in graded alcohol, the samples were submerged in CO_2 and dried with a critical point dryer. Then, the tissue was mounted in aluminum stubs and vacuum coated with a molecular layer of gold. Observations were made under a S-4000 scanning electron microscope (Hitachi, Tokyo, Japan).

For the purpose of collagen observation, scanning electron micrographs were taken at 15 kV and a magnification of mainly ×40,000. Micrographs were printed using a Leitz photo-enlarger on 16 × 12 cm Ilford multigrade black and white paper. Collagen morphometry was performed on the photomicrographs which had a final magnification of ×95,000.

Electron microscopy. Following fixation in glutaraldehyde and after buffering, samples for electron microscopy (EM) were post-fixed in 1% buffered osmium tetroxide, dehydrated and embedded in epoxy resin. The sections of 600–900 nm were then cut with an Ultramicrotome (Om U3, C. Reichert, Austria) and stained with a lead

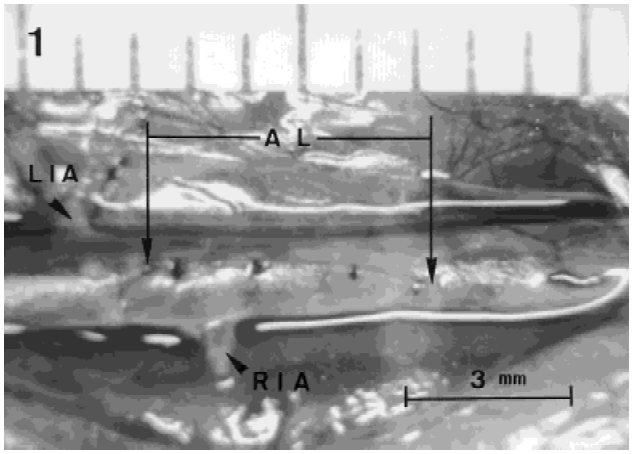

Fig. 1. Anastomotic line (AL) immediately after diode laser welding. Note a slight thickness with blanching and dry flattening on the welding line. LIA, left iliolumbar artery; RIA, right iliolumbar artery.

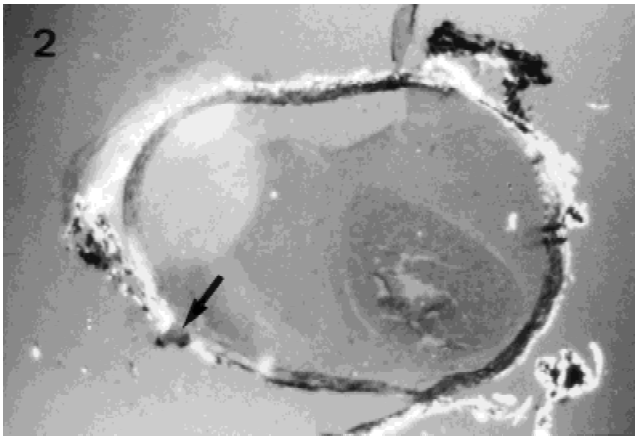

Fig. 2. Transversal section of aorta welded immediately after laser irradiation, stained with picrosirius red and visualized under polarized light. Birefringence of collagen's fibers in the anastomotic site (arrow) has disappeared at a low magnification; ×12.

citrate and 1% uranyl acetate. Sections were examined in a H-7100 electron microscope (Hitachi), calibrated with a diffraction grating replica of 54,800 lines per inch.

For the purpose of collagen measurements, electron micrographs were taken at 75 kV and a magnification of mainly ×60,000, with a small number at ×30,000. Micrographs were printed using a Leitz photo-enlarger on 14.7 × 12.1 cm Ilford multigrade black and white paper. The final magnifications were ×96,000 and ×48,000.

RESULTS

Light Microscopy

After diode laser welding, the anastomotic site showed a slight thickness with blanching and dry flattening observed with the naked eye (Fig. 1). With the light microscope, the two cut edges were fused, but elastic laminae, myocyte layers, and collagenous fibers of the media were disorganized. Optical refringence and structure of collagen fibers at the adventitia disappeared and collagen fibers were coalescent by laser heating. This zone was stained dark red instead of the normal bright red with van Gieson stain, and gray-blue instead of bright blue with Heidenhains' azan stain, as well as becoming completely homogeneous. Such a change of color is significant for damaged collagen [8].

Under polarized light, the collagenous fibers in the region of fusion had a disappeared birefringence when the sections stained with picrosirius red. This zone was about 120–150 μm in width, and extended throughout the vessel wall (Fig. 2). However, with the higher magnification, there were also some collagen's fibers that kept their optical birefringence, especially the ones that were under the elastic fibers (Fig. 3). The latter was confirmed by EM also.

On day 3, the collagen fibers in this region were the same as on day 0, and still remained weak birefringence under LM. The adventitia was thickened, and presented an important inflammatory cell infiltration. On day 10, there were important new collagenous fibers covering the surface of the anastomotic site with small areas of coagulation necrosis at the adventitia. In addition, the edematous, adventitia, with important inflammatory cell infiltration, presented small areas of tissue necrosis and foci of some mononuclear and polymorphonuclear leukocytes located at the point of the junction on the media.

Electron and Scanning Electron Microscopy

After diode laser welding, the welding effect of thermal irradiation was evidenced by a weld of collagen bundles under SEM. In cross-section, the collagen fibers were merging with one another and lost their regularity in outline and size (Fig. 4). Under EM, several different types of changes were observed in the areas where the collagen fibers appeared to fuse (Fig. 5): 1) some fused with each other which could be identified with two different directions of periodicity; 2) some, like rope yarns, intertwined with each other and formed a "rope." These fibers often lost parts of their periodicity; 3) some were swollen and their diameters

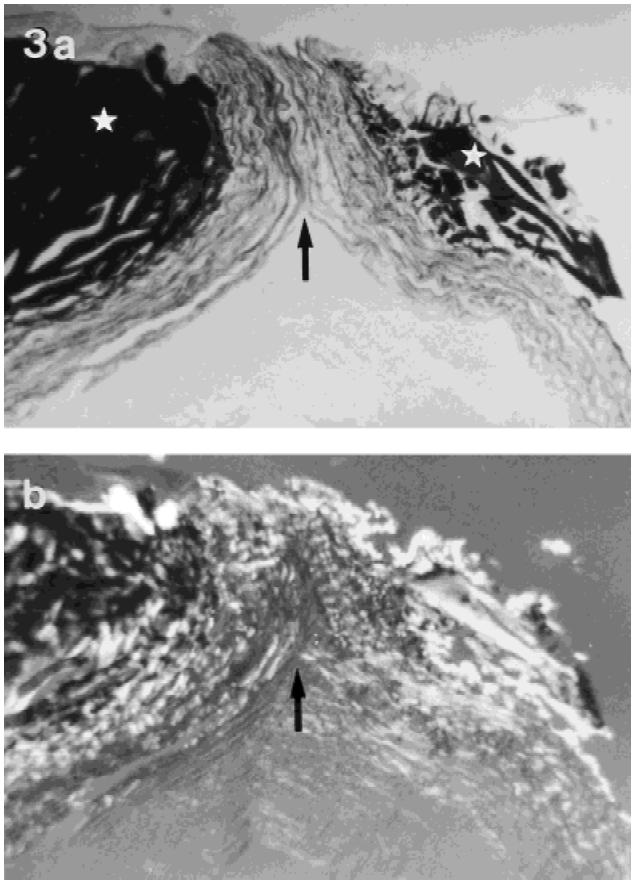

Fig. 3. Microscopic view of anastomotic site (arrows) immediately after laser welding. Sections stain with picrosirius red. Under non-polarized light examination **(a),** optical refringence of collagen fibers disappears in the adventitia (white stars). Under polarized light **(b),** the birefringence of collagen fibers into the media and the adventitia obviously decreases. However, some collagen's fibers, especially under the elastic fibers, still keep their birefringence. Lumen side down; ×320.

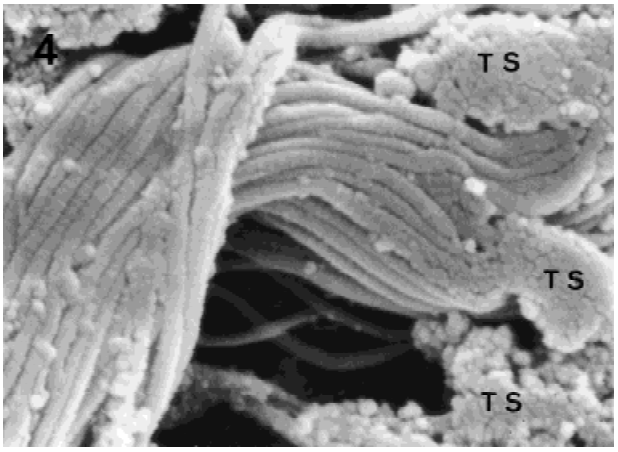

Fig. 4. Welding effect of thermal irradiation evidenced in collagen bundles. Transversal section (TS) of sealed collagen fibers presenting loss of regularity in outline and size. SEM; ×20,000.

varied from 100–160 nm, twice their normal diameter (70–80 nm), however the swollen collagen fibers did not lose their periodicity; and 4) some had been dissolved and their periodicity was completely unrecognizable. All of these changes could be observed in the same zone and were easily found in the samples which were fixed before stopping laser irradiation. All of the involved collagen fibers, that either retained all or part of their periodicity, never changed their distance of periodicity (approximately 55 nm).

On day 3, the areas where the collagen fibers had completely dissolved had increased. It was difficult to find collagen fibers which had changed morphologically as described on day 0. In some regions where the tissue had been practically homogenized, there was evidence of normal collagen fibers presenting clear periodicity (Fig. 6).

On day 10, the most characteristic morphologic change was a large number of new collagen fiber proliferating in the adventitia and the media. The active young fibroblasts and myofibroblast cells, as described by Godlewski et al. [9], were easily found in the adventitia with abundant exocytoses and tropocollagen around them (Fig. 7). In the areas where the tissue was completely homogenized with few inflammatory cells, a large number of newly formed collagen fibers were found at the junction between media and adventitia (Fig. 8).

DISCUSSION

At the present time, the mechanism of laser vessel welding is not completely understood. In this article, the modification of the collagen fibers induced by 830 nm diode laser fusion are presented now.

Under light microscopy and specially with Van Gieson or Heidenhain's azan stains, the denatured collagen bundles appear, respectively, dark red or gray-blue. After laser irradiation, the reduced birefringence could be visualized by picrosirius red stain observed under polarized light. Effectively, the picrosirius red stain greatly enhances the natural birefringence of normal collagen fibers when illuminated by polarized light [10], inversely, does not exhibit birefringence in thermally denatured collagen. Furthermore, the zone of collagen denaturation is well demarcated

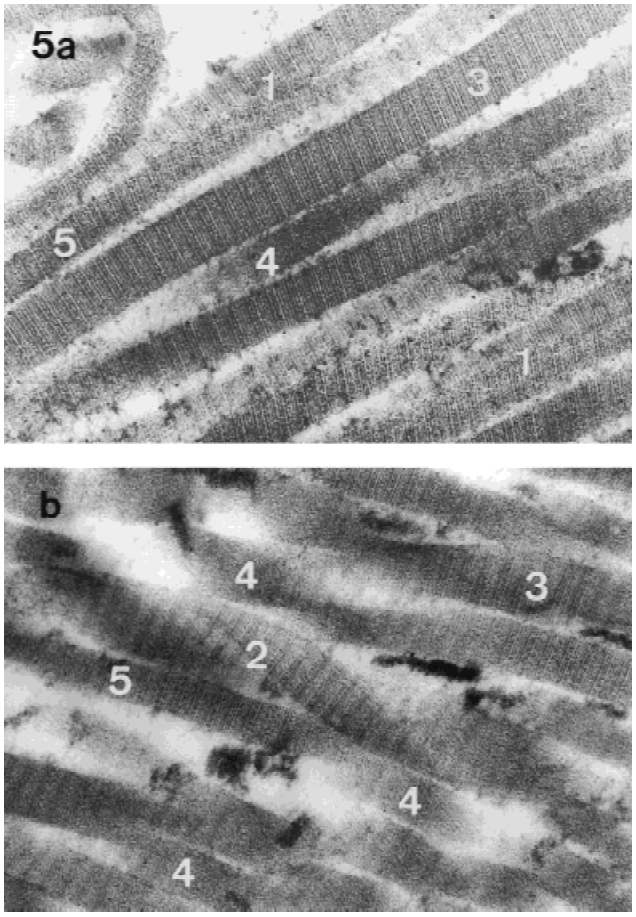

Fig. 5. **(a-b)** Electron microscopic view showing collagen fibers' alterations after diode laser welding. 1) Fused collagen fibers with coalescence of their periodicity. 2) Intertwined and like "rope" collagen fiber. 3) Swollen collagen fibers. Note the normal periodicity of swollen fibers (55 nm). 4) Dissolved collagen fibers with disappearing of their periodicity. 5) Note normal collagen fibers in the same place. EM; ×60,000.

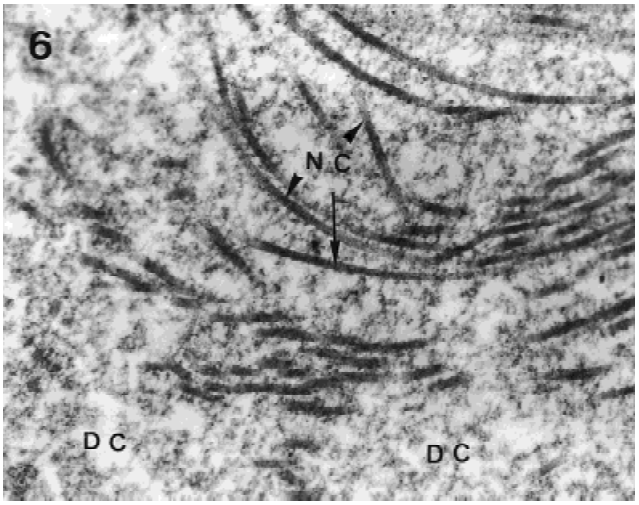

Fig. 6. On day 3, note dissolved collagen fibers (DC), interspersed between normal collagen fibers (NC) presenting clear periodicity. EM; ×30,000.

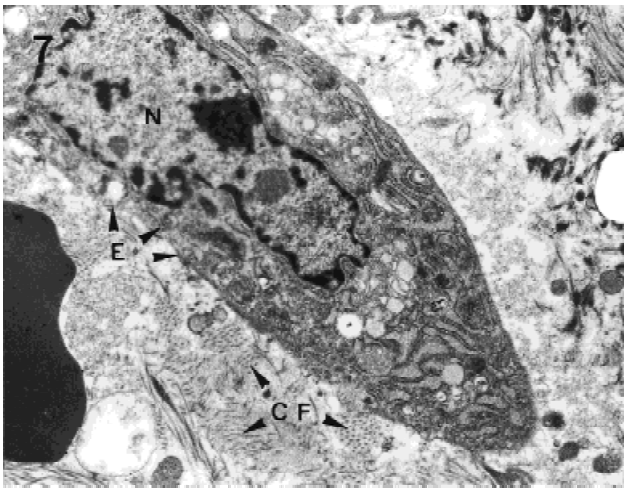

Fig. 7. On day 10, presence of myofibroblast in the adventitia. Note the high cellular activity with abundant exocytoses (E). N, nucleus; CF, collagen fiber. EM; ×7,000.

and can be measured directly by optical micrometry [11]. In our observation, the collagen fibers located in the anastomotic site do not completely lose their birefringence, some of them being thermally respected and not denatured. This finding is confirmed by electron microscopy.

Our morphologic electron microscopy study shows that some collagen fibers fuse with each other after laser welding. This finding confirms Anderson's hypothesis that the welding process occurred as a result of the unraveling of collagen bundles at the cut ends, with partial interdigitation of the bundles across the cut [5,12,13]. However, we consider that crosslinking is not only occurring at the cut ends of collagen fibers, but also between their parallel faces. This fact is of importance, if one considers that the end-to-end sealing of cross-sectioned collagen fibers on the only 0.1–0.2 mm thickness of cut end is not frequently obtained in the laser assisted anastomosis with real microsurgical techniques. Two parallel collagen fibers can be "roped" after laser welding, and this kind of bonding is probably the main type of adhesion in laser fusion.

Because collagen fibers abutting elastic fibers are less changed after diode laser welding, it is difficult to find the phenomenon of collagen fibers to elastic fibers bonding, which was observed by White et al. [2,14].

It is noted that the different types of collagen fibers' modifications observed after laser welding, such as fused, roped, swollen, dissolved can re-

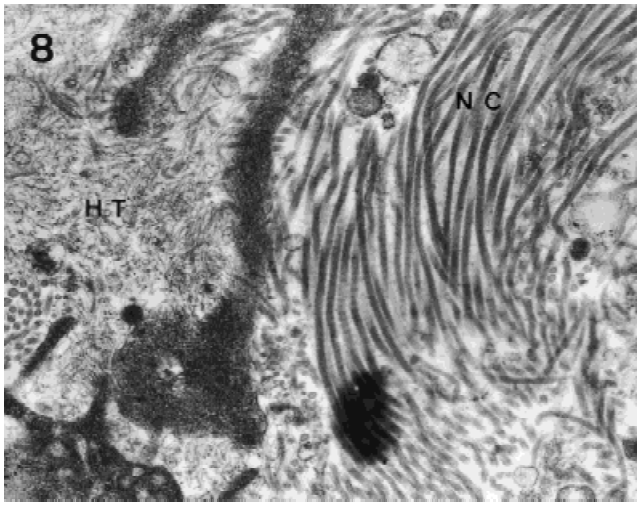

Fig. 8. On day 10, note high density of newly formed collagen fibers (NC) neighboring homogenized tissue (HT) and located at the junction between media and adventitia. EM; ×30,000.

main with normal fibers in the same zone. This means that collagen fibers have different reactions to the same level of temperature. Our previous biochemical study also shows that collagen fibers do not completely denature after laser welding [15]. This phenomenon could be explained by the fact that there are several types of collagen in the vessel wall [16], and that each type of collagen contains different proportions of hydroxyprolin residues with different stability to temperature [17]. Further immunohistological or biochemical studies are needed to determine: 1) which types of collagen are fused and roped, thus playing a role in the laser welding; and 2) which types of collagen fibers retain a normal structure after laser irradiation, thus theoretically stabilizing the vessel wall and preventing pseudoaneurysm formation.

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